Genetic Research and Type 2 Diabetes Mellitus

[26-10-2011]

[Thesis – Drug Innovation Master Program]

[**ABSTRACT:** Type 2 Diabetes Mellitus is a complex metabolic disease with a dysregulated glucose homeostasis. This disease is caused by both environmental and genetic factors. Current treatments aim at relieving the symptoms and slowing down the disease progression, rather than the actual etiology of the disease. Therefore, it is necessary to develop therapeutic strategies that modify the course of the disease and target at the pathophysiology of T2DM. Recent advances in genotyping techniques allow the identifications of more and more genetically related therapeutic targets. In the future, with further research, these targets are believed to contribute to the earlier diagnosis and improved disease managements. It is important to realize that a personalized life-style managements and genetic therapies should be applied to T2DM patients simultaneously and the future T2DM treatments are believed to be no longer a single option, but will tackle several aspects of this disease at the same time.]

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Research Question:

What have studies focusing on the genetic background of Type 2 Diabetes Mellitus contributed to the understanding of the etiology and pathogenesis of this disease until now? Could this knowledge contribute to the development of future therapies and treatments?

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Introduction

Diabetes mellitus (DM), often simply referred to as diabetes, is a group of metabolic diseases that is characterized by a chronically elevated serum glucose level (Malecki., 2005). If diabetes is undiagnosed or poorly controlled, it can lead to a state of persistent hyperglycemia, resulting in complications and irreversible damage in a wide range of tissues, most notably the retina, the kidney glomeruli, neural tissue and blood vessels (Wolfs MGM, 2009). Due to these complications, patients with diabetes often have a reduced life expectancy and quality of life (Zimmet P, 2001). In total, it has been estimated in 2009 that there were more than 200 million patients with diabetes worldwide (Wolfs MGM, 2009).

Although diabetes is most often divided into three main types, namely type 1, type 2 and gestational diabetes, other more rarely seen types of this disease have also been described, such as neonatal diabetes, congenital diabetes, cystic fibrosis-related diabetes and steroid diabetes (Malecki., 2005).

Type 1 diabetes mellitus is an autoimmune disease leading to destroyed pancreatic β-cells with an absolute insulin deficiency as a result; therefore these patients are dependent on exogenous insulin. Gestational diabetes occurs in pregnant women who have never suffered from diabetes, but experience gestational hyperglycemia, which might be transient or develop after the pregnancy into type 2 diabetes mellitus. The focus of this thesis will be type 2 diabetes mellitus (T2DM), which is a condition characterized by both insulin resistance (poor tissue insulin sensitivity) and impaired insulin secretion from the pancreatic β-cells (Petrie JR, 2011)

Epidemiology of T2DM

Comparing to the other two major types of diabetes, T2DM is the most prevalent form and is responsible for 90% of the overall diabetes prevalence (Malecki., 2005). The incidence of the disease has been growing rapidly in the past years. In the western world, T2DM has virtually become epidemic due to the typical western life style of sedentary behavior and high calorie diet. However, rates of diabetes are increasing also in non-Western countries; for example, the greatest percentage increase in diabetes incidence is expected to occur in Africa over the next 20 years (Wolfs MGM, 2009). According to statistics from the World Health Organization (WHO), in 2006, 180 million people have been estimated to suffer from T2DM and the annual death rate due to this disease was 3 million by that time. Both numbers are expected to double over the next 25 years (Wolfs MGM, 2009). An interesting fact about T2DM is that the prevalence of this disease varies widely among various racial and ethnic groups as shown in *figure 1* below (Selvin E, 2011), which is suggestive that a genetic component plays a pivotal role in the disease development.

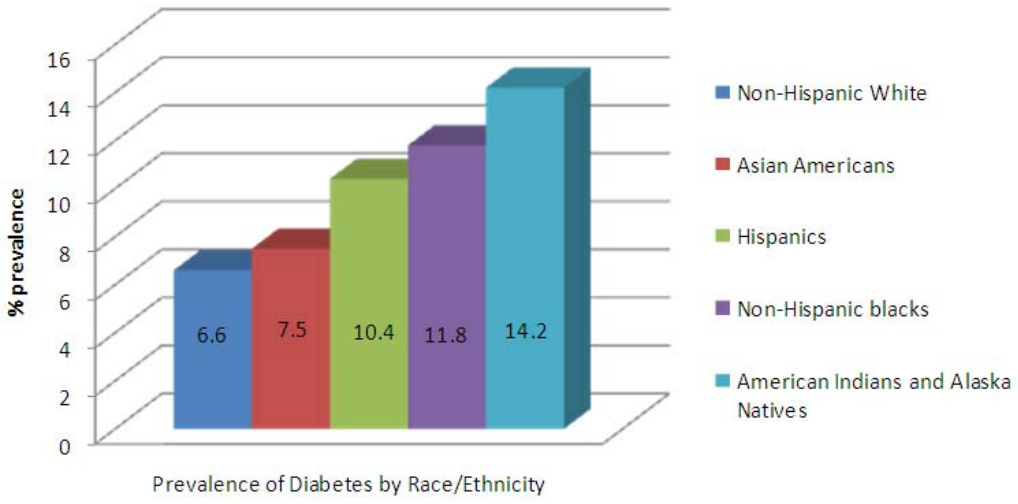

Figure 1. Prevalence of Diabetes by Race/Ethnicity in the United States*. Type 2 diabetes mellitus is more prevalent among Hispanics, Native Americans, African Americans, and Asians/Pacific Islanders than in non-Hispanic whites (Selvin E, 2011).*

Pathophysiology and Risk Factors of T2DM

Type 2 diabetes mellitus is characterized by the combination of disturbances in insulin secretion by pancreatic β-cells (for the molecular mechanism, see *appendix figure 1*) and peripheral insulin resistance, which is often related to obesity (Boden, 1996). Insulin resistance is caused by defects in the signaling pathways that process the insulin signal in its target tissues (Wolfs MGM, 2009). Normally, plasma glucose levels are maintained within a narrow and well-balanced range, known as glucose homeostasis. However, as a consequence of impaired insulin secretion and resistance, glucose uptake and release by pivotal tissues is disturbed, which eventually results in hyperglycemia, as illustrated in *figure 2 (*Boden, 1996*)* below:

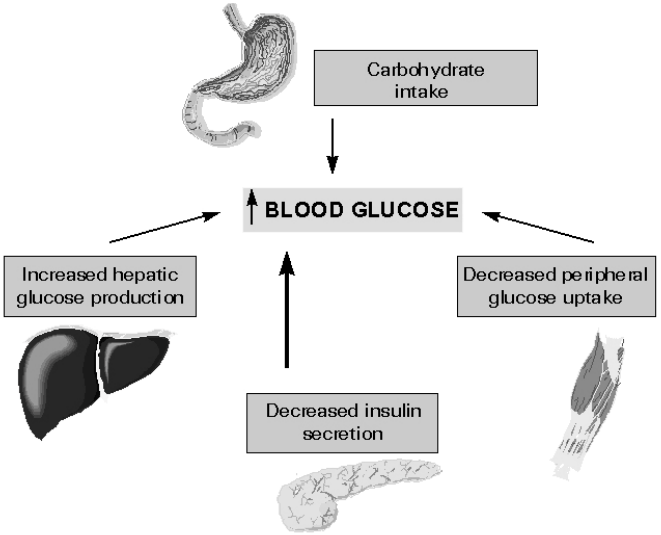

Figure 2: Simplified scheme of pathophysiology of T2DM*. As a combination of impaired insulin secretion and resistance, the effects are often associated to elevated levels of free fatty acids in plasma, decreased glucose transport into muscle cells, increased hepatic glucose production and increased breakdown of fats. All the defects as a whole result in increased blood glucose level in the end (Boden, 1996).*

It has often been suggested that the disease starts with insulin resistance and is followed by increased insulin production by the pancreatic β-cells to maintain glucose homeostasis. At a later stage, due to the long-term compensation mechanism by the β-cells to

keep up with the higher insulin demand, these cells eventually undergo further damage and apoptosis. When the ultimate demand of insulin release cannot be satisfied, higher plasma glucose levels are the result. The vulnerability of the β-cell pool in insulin-resistant conditions is determined by problems related to at least one of the following pathways involved in insulin secretion: β-cell regeneration, β-cell survival, or β-cell development (Wolfs MGM, 2009). It has been shown that although obesity is a major risk factor for diabetes (around 50% of the patients with diabetes are obese), a significant proportion of type 2 patients are not obese (Pimenta W, 1995). Therefore, it has been concluded that although obesity is a major risk factor for developing T2DM, it is the vulnerability of the β-cell pool which determines whether obesity in fact triggers the development of T2DM.

The existence of both insulin resistance and less insulin production is necessary for T2DM to occur. As the disease progresses, microvascular (such as neuropathic complications and kidney damage) and macrovascular (such as coronary artery disease, cerebrovascular disease or peripheral arterial disease) complications can appear. Hyperglycemia seems to be the main determinant of microvascular problems; whereas macrovascular risks are associated with insulin resistance. This is in line with the 'ticking clock' hypothesis of complications suggested by Stern in 1996 (Stern, 1996) and Haffner and Dágostino in 1999 (Haffner SM, 1999): taking T2DM development as a ticking clock, it starts ticking for microvascular threat at the onset of hyperglycemia, while for macrovascular complications, the clock starts ticking presumably with the onset of insulin resistance at some antecedent point. Moreover, metabolic complications including lipid abnormalities might be related to both hyperglycemia and insulin resistance.

Type 2 diabetes mellitus is an extremely complex disease and its development and progression involve but are not limited to the following risk factors: genetics/family history and pre- and post-natal environmental factors (Jin, 2009). Genetic/family history results in certain patterns of DNA sequence whereas pre and post-natal environmental factors include a wide range of elements: suboptimal intrauterine environment, low birth weight (LBW), obesity, inactivity, gestational diabetes and advancing age (Jin, 2009). These pre- and postnatal environmental factors can impact development of key tissues in metabolic homeostasis, which contribute to progression of insulin resistance and β-cell dysfunction. *Figure 3* (Jin, 2009) below summarizes the risk factors and their potential role in the pathogenesis of T2DM. Moreover, unraveling the complex pathophysiology of T2DM is complicated by the secondary effects of 'glucolipotoxicity' (hyperglycemia and hyperlipidaemia) (Freeman H, 2006).

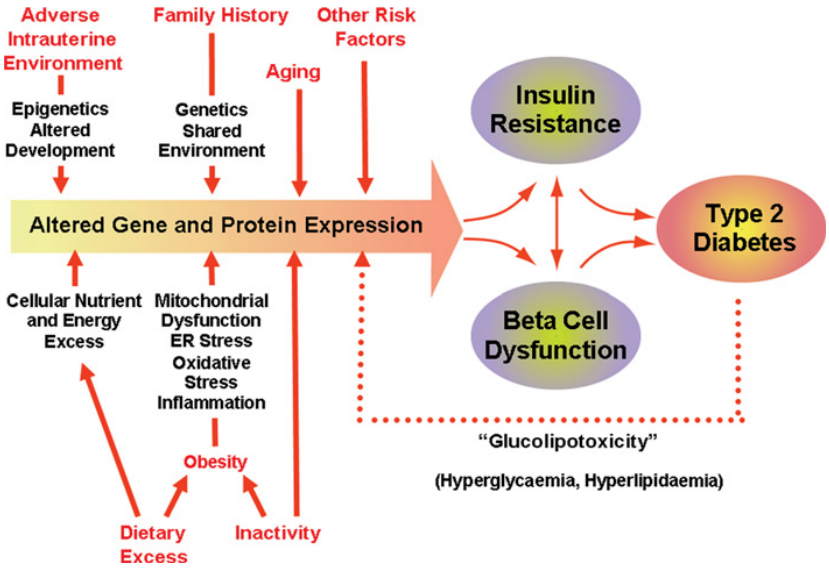

Figure 3: Multiple risk factors involved in T2DM pathogenesis*. Both classical genetic risk (family history) and multiple environmental risk factors contribute to the pathogenesis of T2DM (10). Family history influences the genetic environment of an individual and it may contribute to specific alterations in systemic physiology distinct from other risk factors. Intrauterine environment during critical windows of development including fetal and/or early postnatal period influences the susceptibility to diabetes via epigenetic regulation and obesity during adult life. Adipose tissue is a very important endocrine tissue, which is highly associated with insulin resistance. The capacity of adipocyte to replicate, differentiate and store lipids declines with age, which makes T2DM and obesity age-related diseases. Exercise is critical for maintenance of muscle oxidative function and systemic insulin sensitivity. Individuals with obesity and T2DM have impaired oxidative capacity and increased lipid content in all muscle fiber types. (Jin, 2009) (Lazar, 2005) (Manson, 1991)*

Diagnosis and Current Treatment of T2DM

The diagnosis of T2DM is based on the presentation of classic symptoms and signs including polyuria, polydipsia, fatigue and weight loss; other symptoms that might suggest hyperglycemia include blurred vision, extremity paresthesia or yeast infection (particularly balanitis in men) (Freeman H, 2006). The disease can be accompanied by hypertension, dyslipidemia and polyphagia. To diagnose the disease, biochemical and diagnostic tests are applied. Currently available biochemical tests are blood glucose or urine glucose measurements, blood HbA_{1c} or blood fructosamine measurements; whereas the diagnostic tests include the measurement of fasting plasma glucose (FPG) and/or an oral glucose tolerance test (OGTT) using standard criteria (WHO, 2003). Patients with T2DM are often asymptomatic and their disease can remain undiagnosed for many years. It has been shown by some studies that at the time of diagnosis, the typical patients with T2DM have had diabetes for at least 4-7 years (Glamoclija Una, 2010). Among patients with T2DM, 25% are described to already have retinopathy, 9% neuropathy and 8% nephropathy at the time of diagnosis (Glamoclija Una, 2010). For the healthy population, screening tests and questionnaires might be utilized for risk assessment. Each screening test needs a designated and pre-determined threshold or 'cut-point' that defines high risk.

Currently, there are seven major classes of oral pharmacological agents available to treat T2DM: sulfonylureas (e.g. glibenclamide), meglitinides (e.g. nateglinide), biguanides (e.g. metformin), thiazolidinediones (e.g. pioglitazone), α-glucosidase inhibitors (e.g. acarbose), DPP-IV inhibitors (e.g. sitagliptin) and GLP-1 agonists (e.g exenatide) (Glamoclija Una, 2010).

Sulfonylurea derivatives are insulin secretagogues and function by closing the ATP-sensitive potassium channel (K_{ATP}) of ß-cells leading to more (or prolonged) insulin secretion (Evans JL, 2010) (see *figure 4*).

Meglitinides are a novel class of non-sulfonylurea insulin secretagogues which work by stimulating first-phase insulin release in a glucose-sensitive manner (Evans JL, 2010).

Biguanides have a gluco-regulatory effect only in the presence of endogenous insulin by decreasing endogenous glucose production and reducing peripheral insulin resistance by approximately 20-30% (Evans JL, 2010).

Thiazolidinediones act as agonists for the peroxisome proliferator-activated receptor γ (PPARγ) leading to insulin sensitization and decrease of glucose and lipid levels.

α-glucosidase inhibitors are inhibitory enzymes that block the enzymatic degradation of complex carbohydrates in the small intestine (Evans JL, 2010). These compounds lower post-prandial glucose and improve glycemic control without the risk for weight gain or hypoglycemia (Evans JL, 2010).

DPP-IV (dipeptidyl peptidase-4) inhibitors and GLP-1 (glucagon-like peptide-1) agonists are new classes that are recently launched into the market as novel T2DM treatments (Ahren B, 2004). Glucagon-like peptide-1 (GLP-1) is an incretin hormone with antidiabetic action through its ability to stimulate insulin secretion, increase beta cell neogenesis, inhibit beta cell apoptosis, inhibit glucagon secretion, delay gastric emptying and induce satiety (Ahren B, 2004). Therefore, it is considered to be a novel T2DM treatment. However, a problem is that GLP-1 is rapidly inactivated by the DPP-4 enzyme, which results in a short circulating half-life of the active form of GLP-1 (< 2 min) (Ahren B, 2004). Therefore, currently prescribed GLP-1 agonists haves an optimized molecular structure with extended half-life; while DPP-IV inhibitors inhibite the breakdown of GLP-1 by DPP-IV.

According to European Association for the Study of Diabetes (EASD) guidelines, metformin is the initial choice of pharmacotherapy for T2DM; whereas sulfonylurea agents are the second choice if metformin is not effective (Campbell, 2009). Eventually, when the disease progresses and (the combination of) oral agents are not able to maintain glucose homeostasis anymore, the administration of insulin by injection or pump therapy is the current treatment of choice.

Up until now, the usual diabetes management is confined of lifestyle measures (diet and exercise) together with the treatments mentioned above, with the aim to slow down the disease and its complications. None of the above treatments can restore normal glucose metabolism. Hence, neither changes in lifestyle nor the use of medication are sufficient to target the etiology of this disease or to modify the disease pathophysiology, not even to mention a cure. This leaves the patients open to risks of life-threatening complications and substantially decreased life expectancy rate and quality of life; meanwhile, this disease causes tremendous burdens both socially and economically. Thus, it is necessary to search for alternatives to the oral pharmacological agents and limited existing interventions mentioned above and to target the diabetic pathways in a cellular/molecular way. In addition, it seems to be more effective if lifestyle changes, medication or both are provided early to T2DM patients (Wolfs MGM, 2009); therefore, improvements of the early diagnostic techniques and the opportunities for early intervention will greatly improve the effects of current ways of managing type 2 diabetes.

Identification of the molecular genetics of T2DM opens the possibility of understanding the genetic architecture of clinically defined categories of diabetes, new biological insights, new clinical insights, and new clinical applications (McCarthy MI, 2008). Therefore, further investigation into the basis of the genetic traits (e.g. ß cell dysfunction and insulin resistance) of T2DM is of vital importance, as such knowledge could lead to the discovery of new therapeutic targets that will modify the course of the disease and can be utilized as a long-term T2DM treatment.

In short, the aim of this thesis, which is to answer the research question, lies on encapsulating the potential therapeutic targets of T2DM by looking into the current knowledge of the genetics of T2DM. These genetic targets are evaluated and their future perspectives are discussed.

Methods/Strategies Applied in Gene Studies of T2DM

The genetic component of T2DM can be well supported by the following knowledge: i) inheritance seen in families with rare monogenic forms of diabetes (Freeman H, 2006); ii) high prevalence of the disease in particular ethnic groups and its modification by genetic admixture (Florez JC, 2003); iii) the difference in concordance rates between monozygotic (concordance rates of over 90%) and dizygotic twins (concordance rates of 9-16%) (Wolfs MGM, 2009); iv) results from numerous linkage studies. Monogenic forms of diabetes account for up to around 5% of T2DM (Vionnet N, 2007), but most cases of T2DM do not show clear Mendelian inheritance patterns. In fact, T2DM is seen as a genetically complex disorder, in which genetic variants predispose individuals to develop the disease. Moreover, environmental factors play a substantial role by interacting with the genetic variants, which make the genetic studies into the disease susceptibility and progression rather difficult.

Recent advances in genotyping techniques and the collection of large, T2DM patient cohorts have considerably contributed to the development of T2DM genetic studies from the old fashioned candidate-based association studies. These advanced genotyping techniques include genome wide association studies using high-density single nucleotide polymorphisms (SNPs); and microarray approaches (Vionnet N, 2007).

Candidate-Based Association Studies

The candidate-based association studies can be partially linked to human evolution theory that in the present era of food excess, the genes that have been conserved for promoting efficient food collection and nutrient storage during periods of famine would nowadays be disadvantageous and may contribute to energy storage, impaired energy expenditure and the risk of T2DM (Speakman. 2007).

Association studies investigate the relationship between disease and genetic markers, comparing the diabetic patients with a healthy, non-affected population. Several association studies have been performed to test some of the genetic variants in a gene that is a strong candidate for being involved in the disease. Although a few genes have been identified in this way (as mentioned below), in general these studies have not been very successful. The few diabetes genes that were identified and replicated via association studies had already been linked to a diabetic phenotype, since the first step of this study is a selection of the gene based on its biological function and potential relationship with diabetes, such as insulin secretion or action in muscle, adipose tissue or liver (Jin, 2009).

The next step is to search for a mutation, usually by direct sequencing of the coding and regulatory parts of the gene. After the mutation has been identified, a verification procedure is carried out, which includes the following:

1) verification of the co-segregation of the mutated sequence with the disease in linked families; 2) confirmation of the absence of the mutation in unrelated healthy groups; 3) identification of the biological role of the identified sequence differences in biological experiments (McCarthy, 2002).

A key example is the gene encoding PPARγ (PPARG) initially identified as a nuclear receptor key for adipogenesis (Rosen ED 1999). Mutations in this gene can lead to insulin resistance, hypertension and lipodystrophy (Jin, 2009). Similarly, genes influencing β-cell function have been identified as T2DM-susceptibility genes. For instance, the E23K polymorphism in the ATP-sensitive potassium channel (see *figure 4*) Kir 6.2 (KCNJ11) is consistently associated with disease risk as a result of reduced insulin secretion (Jin, 2009). Moreover, mutations in KCNJ11 and insulin (INS) genes have found to be related to permanent neonatal diabetes mellitus (PNDM) (Stoy J, 2007).

Positional approaches to candidate gene identification have also been unsuccessful, with the exception of the genes calpain 10 (CAPN10) (Horikawa Y, 2000) and transcription factor 7-like 2 (TCF7L2) (Grant SF, 2006). Details of these two genes will be explained later on.

Genome-Wide Association Studies (GWAS)

Different from candidate-based association studies, GWAS is a hypothesis-free approach since nothing is assumed about the physiology of the putative gene (Malecki., 2005). Hence, it is a genome scan approach that is less biased regarding presumed functions or chromosomal locations. These studies are looking for linkage between a trait under investigation and a given chromosomal location via the usage of specific laboratory and analytical methods (Malecki., 2005). This linkage indicates the probability of co-segregation of a disease with a specific chromosomal locus. To search for linkage of Mendelian traits, the geneticists usually apply the method called parametric linkage analysis, which a detailed description of this method is review by McCarthy et al (McCarthy, 2008).

Genome-wide association studies typically involve the assessment of the association of single nucleotide polymorphisms (SNPs) with a disease or with a so-called 'phenotypic trait', i.e., type 2 diabetes. It should be noted that GWAS identify association of a genetic locus, a haplotype (A haplotype means the alleles represented by SNPs that are close together stay on the same chromosome in further generations), but not directly of a gene (Wolfs MGM, 2009). Moreover, it is also possible that even when a SNP is in a gene, it can influence the expression of a nearby gene located several thousand base-pairs or more away (McCarthy MI, 2008). Therefore, it is sometimes difficult to determine which gene is responsible for the association signal in a GWAS with full certainty (Wolfs MGM, 2009). Once a gene or gene locus is defined, a verification process must be carried out as mentioned earlier. At the moment, 38 individual susceptibility loci for T2DM have been identified by genome wide association studies (Petrie JR, 2011).

There are limitations of this approach, primarily with respect to study design and detection of control and diseased individuals. For example, a healthy individual may have significant insulin resistance, yet be misclassified as non-diabetic if thorough metabolic analysis is not performed (Jin, 2009). However, in general, GWAS have been quite successful for the genetic studies of T2DM since several novel common genetic variants associated with a diabetic phenotype have been either identified or confirmed with this approach.

As an example, the linkage of transcription factor TCF7L2 to T2DM risk was firstly discovered by using a positional approach, but this finding was rapidly supported by identification of the gene as high-ranking in GWAS as well. Studies have shown that homozygosity for this high-risk allele confers approximately a 2 fold higher risk of T2DM (Grant SF, 2006). It was suggested that the increased risk of T2DM linked to TCF7L2 polymorphism could be due to its effects on islet expression and incretin-mediated secretion leading to impaired glucose tolerance in the end (Lyssenko V, 2007). Moreover, this gene is also expressed in the hypothalamus and plays a major role in the Wnt signaling pathway and development (Lyssenko V, 2007). Additional novel T2DM susceptibility loci were identified and the list can be found in the appendix.

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In the near future, it is believed that more and more genes and loci that are associated with the disease phenotype; a large part of genetic variation that confers susceptibility to T2DM will be unveiled. This can be achieved by including more cases to increase the statistical power of GWAS; performing GWAS in wider ethnic groups so to improve the genomic coverage of GWAS and by carrying out meta-analysis of the studies published so far. Moreover, conducting deep sequencing of the associated region could lead to variants with higher odds ratios being identified (Wolfs MGM, 2009).

Microarray Approach

In addition to the two methods mentioned above, microarray approaches have also been utilized to identify candidate genes and pathways dysregulated in islets from humans with T2DM (Jin, 2009). For example, in one study employing isolated islets, the dominant pattern observed was marked down-regulation of expression of the transcription factor ARNT (aryl hydrocarbon receptor nuclear translocator)/HIF-1ß (hypoxia-inducible factor-1ß),

accompanied by altered expression of genes related to glucose sensing and transcriptional control (Gunton JE, 2005).

Genetics of T2DM

Type 2 diabetes mellitus has been empirically shown to be a partially inheritable disease, in which a genetic component plays a significant role in disease etiology. In addition to what has been mentioned in the introduction, a review in 2005 stated that the concordance rate of T2DM for monozygotic twins has been estimated to be much higher than the concordance rate in first-degree relatives (Hansen L, 2005). Some studies have also demonstrated that in T2DM, insulin-stimulated glucose uptake and glycogen storage of skeletal muscles is an inherited impairment (Hansen L, 2005). For instance, biochemical analysis of key enzymes in skeletal muscles, such as glycogen synthase and protein phosphatase-1(PP1) and nuclear magnetic spectroscopy analysis of insulin-stimulated glucose metabolism *in vivo* have illustrated that the typical pathophysiological features of T2DM were present also in the glucose-tolerant first-degree relatives (Hansen L, 2005). Altogether, observations from genetic studies have been widely acknowledged as evidence for genetic components in T2DM, which consists of various forms.

Insulin Deficient Monogenic Forms

There are different types of monogenic forms of T2DM and the most frequent form is characterized by severe impairment in insulin secretion; it is inherited in an autosomal dominant way. Its early onset form is called maturity onset diabetes of the young (MODY), which was used for the first time by Tattersall and Fajans in 1975 (Tattersall RB, 1975). Autosomal dominant traits are characterized by their occurrence in a family, in several subsequent generations, equal frequency in both genders, and the transmission of the disease by men and women (Malecki., 2005). In addition to an autosomal dominant mode of inheritance, MODY is characterized by a high phenotypic penetrance rate, early disease onset (usually by the 20^{th} or 30^{th} year of age), lack of obesity, as well as biochemical and clinical features of impairment in insulin secretion (Mitchell S, 2002). In fact, MODY pedigrees have sometimes been misdiagnosed as type 1 diabetes families due to the sharing of typical clinical features of type 1 diabetes: young age of onset, normal body weight and insulinopaenia (Hansen L, 2005).

Until now, six MODY genes have been identified, they include: hepatocyte nuclear factor-4α (MODY1), -1α (MODY3), -1ß (MODY5) (HNF04α, -1α, -1ß), glucokinase (MODY2), insulin promoter factor-1α (IPF-1α) (MODY4), and NEUROD1 (MODY 6) (Malecki., 2005) (see *figure 4*). Except glucokinase, the other five proteins are transcription factors, which can regulate the expression of insulin genes directly or indirectly by binding to the promoter region of the DNA sequence. Moreover, clinical signs of diabetes associated with transcription factors differ from the glucokinase form (Fajans SS, 2001). However, even among the functions of the transcription factors, there is certain degree of heterogeneity. Patients with MODY3 present better responses to sulphonylurea treatment comparing to patients with other MODY types (Fajans SS, 2001). Insulin promoter factor-1α (MODY4) and NEUROD1 (MODY6) related diabetes differs from HNF04α, -1α, -1ß (MODY1,3,5) associated diabetes in some pathophysiological aspects (Fajans SS, 2001). The common functional feature of IPF-1α and NEUROD1 proteins is a direct interaction with the most active part of the insulin promoter, so called mini-enhancer (Fajans SS, 2001).

Unlike the transcription factor diabetes with relatively small pathophysiological and clinical differences between subjects, the glucokinase[1] form usually presents more differences among subjects (Frayling TM, 2001). The function of glucokinase is essential for the energy production process and subsequently for ATP synthesis and insulin secretion as it is a rate limiting enzyme of glucose metabolism, which acts as glucose sensor in pancreatic ß-cells (Malecki., 2005) (see *figure 1*, appendix). Glucose metabolism abnormalities (e.g. impaired

[1] Glucokinase is a key regulatory enzyme of the pancreatic cells that catalyses glucose phosphorylation to glucose-6-phosphate (Malecki., 2005).

fasting glucose) are present in early childhood and they are usually stable afterwards during the rest of one's life with glucokinase-related T2DM. This monogenic type usually shows a modest phenotype and its chronic form often does not lead to further complications (Frayling TM, 2001), which is probably due to the simple mechanisms related with glucokinase mutations. On the other hand, transcription factor diabetes has much more complicated biological mechanisms that involve the process of ß–cell development and expression of many other genes, which results in a more severe phenotype.

It is an interesting fact that all the diabetogenes identified including the ones mentioned above are essential for the development and appropriate functioning of the pancreatic ß–cells. *Figure 4* (Graeme IB, 2001) below summarizes the relation between pancreatic ß–cells function/dysfunction and the six MODY genes.

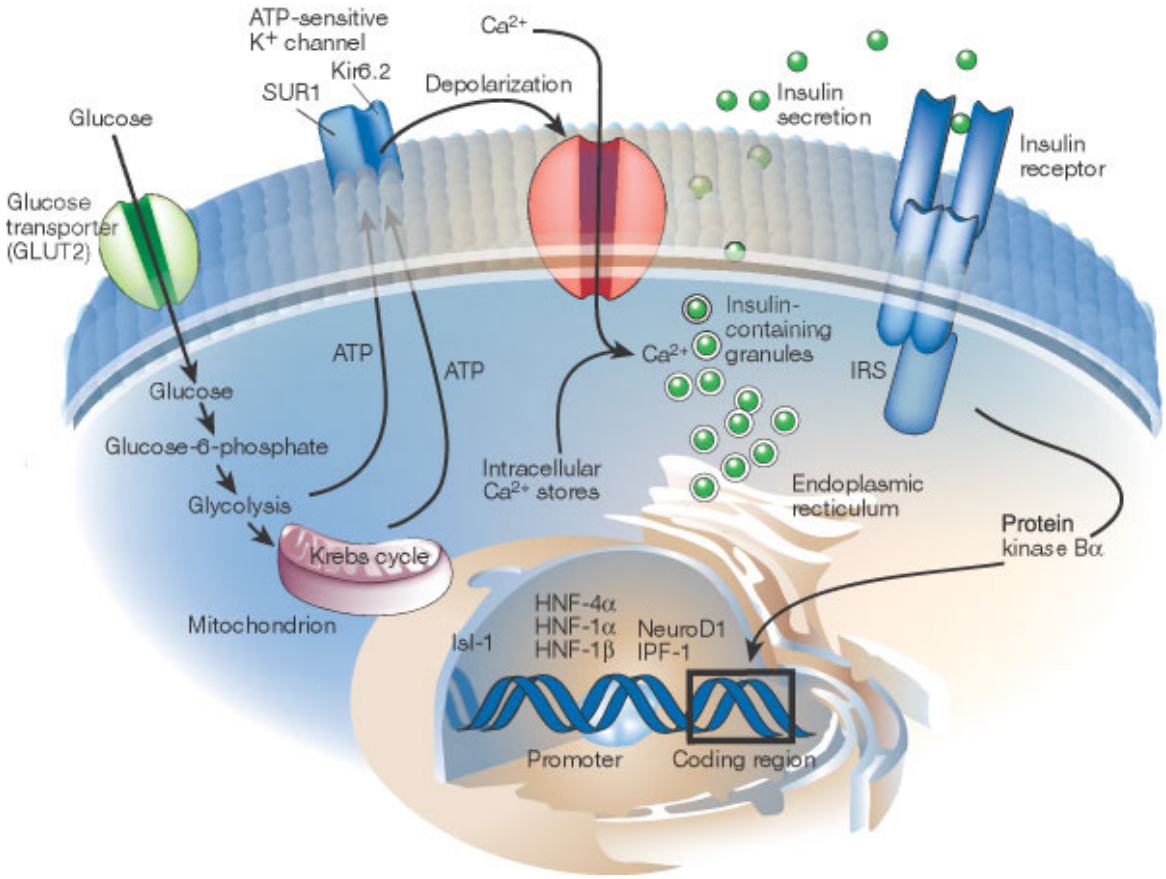

Figure 4: The relation between β-cell and MODY genes in T2DM. *Glucokinase (MODY2) functions as the glucose sensor of the β-cell by catalyzing the transfer of phosphate from ATP to glucose to form glucose-6-phosphate following the transport of glucose into the β-cell by the glucose transporter 2 isoform (GLUT2). Processes including glycolysis and the Krebs cycle generate ATP leading to the closure of the ATP-sensitive K^+ channel — a hetero-octamer comprised of four subunits of the sulphonylurea 1 receptor (SUR1) and four subunits of the inwardly rectifying K^+ channel Kir6.2. Mutations in these proteins are associated with familial persistent hyperinsulinaemia hypoglycaemia of infancy. Following the closing of the ATP-sensitive K^+ channel are plasma membrane depolarization and extracellular calcium influx, which together with intracellularly released calcium lead to fusion of insulin-containing secretory granules with the plasma membrane and the release of insulin into the circulation. The pancreatic β-cells have insulin receptors and there is evidence for an autocrine action of insulin on β-cell function, including transcription of the glucokinase and insulin genes. The MODY-associated transcription factors HNF-4α (MODY1), HNF-1α (MODY3), HNF-1β (MODY5), IPF-1 (MODY4) and NeuroD1 (MODY6) regulate the transcription of insulin and other β-cell genes. Mutations in islet-1 (Isl-1) may also lead to β-cell dysfunction. Protein kinase Bα may be important in determining β-cell mass (Graeme IB, 2001).*

In addition to MODY, another monogenic form of T2DM defined on the molecular level is maternally inherited diabetes, which is a consequence of mutations in mitochondrial DNA. The maternally inheritance occurs because only maternal mitochondrial DNA is

transmitted to the offspring. Frequently, this type of T2DM is referred to as MIDD (maternally inherited diabetes with deafness) due to the often associated hearing abnormalities. Many mutations have been found in mitochondrial DNA with diabetic phenotype, but the most frequent one is the A3243G substitution in the leucine tRNA gene (Wojewoda M, 2011). Diabetes that results from this mutation may be a part of MELAS syndrome (mitochondrial encephalopathy, lactic acidosis/myopathy, and stroke-like episodes). The mechanism of this mitochondrial mutation on glucose homeostasis is probably associated with the impairment of glucosensory function of the β-cells as well as with its decreased ability to produce insulin, which can be confirmed with clinical studies: pancreatic islets atrophy and reduction in the number of β-cell in patients with MELAS syndrome have been revealed during autopsy examinations (Wojewoda M, 2011). Therefore, a compromised mitochondrial metabolism may indeed be a primary pathogenic event in the β-cells, leading to defective insulin secretion. So it is possible to suggest that the genes encoding mitochondrial proteins are an emerging group of candidate genes not only for relatively rare monogenic forms of diabetes, but certainly also for the more common form of type 2 diabetes (Hansen L, 2005).

Several different mechanisms involved in insulin gene mutations can lead to the development of extremely rare monogenic T2DM. Firstly, mutations can occur in the binding sites of the insulin gene promoter, for example, IPF-1α mutation can cause a decrease in mRNA synthesis, and subsequently impairment in insulin secretion (Malecki, 2003). Secondly, in some rare cases, the mutation could affect the coding region of the gene, which changes the amino acid structure of the hormone and leads to its decrease or even abolished function (Malecki., 2005). For that reason, it is possible that even when the level of the insulin measured in T2DM patients is elevated, the actual amount of the active hormone is substantially decreased since the majority of the circulating polypeptide is an inactive form. Finally, mutations in the binding site of endopeptidase that transforms proinsulin into insulin has been described (Malecki., 2005). The carriers of these mutations show signs of hyperproinsulinemia, but the level of normal, matured insulin is decreased (Malecki., 2005).

Insulin Resistant Monogenic Form

As mentioned earlier, T2DM is characterized by insulin resistance and decreased insulin production. The monogenic genes that have been discussed above are all insulin-secretion related. Despite of the important role of insulin resistance in the development of T2DM, its working mechanism at a molecular level has not been revealed to a large extent. A well known insulin resistance form is associated with insulin receptor (IR) mutations. However, mutations in one or two alleles of IR genes that are responsible for the monogenic form of T2DM are very rare. The mutations usually occur on several functional levels: pro-receptor abnormalities, impairment in the IR transport on the cell surface, decreased ability of binding with insulin, or low tyrosine phosphatase activity (Freeman H, 2006).

Recently, two more genes were found to be related to rare phenotypes of extreme insulin resistance and subsequent diabetes. It has been suggested and discussed for many years that a post-receptor defect in insulin signaling can lead to severe decrease in insulin sensitivity. A mutation in AKT2 gene, which encodes the serine/threonine kinase that is highly expressed in insulin-sensitive tissues is responsible for the clinical picture including lipodystrophy (George S, 2004). In addition, the PPARγ gene encoding a transcription factor from a nuclear receptor family, which binds to specific DNA sequences (PPARγ responsive elements) in the promoter of other genes and influences their expression, is another gene found to be connected with the insulin resistant monogenic T2DM (Malecki., 2005). Based on its mechanism, PPARγ is also essential for insulin action and glucose homeostasis. Rare mutations in this gene result in the syndrome of severe insulin resistance and subsequent T2DM that is accompanied by partial lipodystrophy (absence of fat in the limbs and gluteal region), lipid abnormalities (high triglycerides, low HDL cholesterol), hypertension and hepatic steatosis (Savage DB, 2003).

Studies looking into the common genetic variants associated with insulin resistance and obesity suggests that ENPP1 mediates both insulin resistance while concurrently being involved in both obesity and T2DM development (Meyre D, 2005). This discovery supports the idea that comparable molecular mechanisms underlie both conditions. It has been realized that genetic variants of ENPP1 influence the amount of circulating protein and a resulting increase in ENPP1, which could impair insulin binding to its receptor in muscle and brain leading to fat deposition (Meyre D, 2005). The association of ENPP1 with insulin resistance and earlier onset T2DM has been supported by three publications in different populations by 2006 (Freeman H, 2006).

In addition to the application of human genetic studies, biological and physiological studies of models have also provided us some candidates that strongly associate weight gain with the onset of insulin resistance. The research group Yang *et al.* (Yang Q, 2005) report the retinol binding protein-4 (RBP4), a fat derived peptide, can impair insulin sensitivity systemically by modulating glucose homeostasis using five independent mouse models of obesity and insulin resistance as well as obese humans. They identified that when RBP4 is elevated, systemic insulin resistance is observed and when reduced, insulin action is improved (Yang Q, 2005). Many research groups have suggested the possible mechanism of RBP4, which decreases the activity of PI-3 kinase and the phosphorylation of insulin receptor substrate-1, both effects clearly suggesting impaired insulin action (Freeman H, 2006). Interestingly, human patients' studies show that elevated serum RBP4 is correlated with insulin resistance in subjects with obesity, impaired glucose tolerance or T2DM, and non-obese, non-diabetic subjects with a strong family history of T2DM (Graham TE, 2006). This raises the possibility that RBP4 levels could be used for risk of assessment of T2DM and further that RBP4 may play a causal role in insulin resistance and represent a promising therapeutic target.

Complex T2DM

While the genetic studies of MODY have been successful as mentioned earlier, studying the genetics of other forms of T2DM has been difficult due to the complexity of this disease. The following difficulties partially explain the reason of the slow progression of the genetic discoveries of complex T2DM: i) late onset of the disease; ii) increased morbidity and mortality resulting in incomplete pedigrees consisting of no more than two generations; iii) huge variations in phenotypes; iv) clinical and pathogenic heterogeneity; v) absence of a clear transmission pattern within the pedigrees (Hansen L, 2005). However, through years of efforts, a few of the discovered genes were fully proven to influence the susceptibility to this complex disease. It is worth mentioning that this is not the same as a susceptibility gene. The term 'susceptibility gene' implies that a gene is not strong enough to act like a diabetogene on its own, but can only act as a pro-diabetic gene by interacting with other pro-diabetic genes, the metabolic environment of the body (e.g. glucotoxicity and lipotoxicity) and the life style (e.g. sedentary life, excess calories, smoking, stress and chronic inflammation) (Hansen L, 2005).

It took several years of intensive searching to identify calpain 10 as a gene responsible for linkage of T2DM with a region on chromosome 2q in Mexican-American population (Horikawa Y, 2000). The importance of this gene in the T2DM pathogenesis is population dependent. For instance, calpain 10 seems to be partially responsible for about 40% of T2DM occurrence in Mexican-Americans; whereas this value is much lower in a British population (Horikawa Y, 2000). The exact molecular mechanism of this gene remains to be revealed, though it is likely to involve both an increase in insulin resistance and insulin secretion impairment by participating in the breakdown of other proteins and thus, through its proteolytic function, it can modulate the activity of other enzymes, and also modify the apoptosis process (Sreenan SK, 2001). In fact, the risk of T2DM development appears to be associated with a haplotype created by three SNPs: -19, -43, -63 rather than with a variant of a single polymorphism. All those SNPs are localized in introns, and thus do not influence the

amino acid structure of the proteins, but more likely to be involved in the pathophysiological mechanisms of calpain 10 (Horikawa Y, 2000).

Fascinatingly, gene mutations that are involved in monogenic diseases are excellent candidates for the search of frequent polymorphisms that predispose to polygenic disease forms. For example, Pro12Ala amino acid variant, a frequent polymorphism in PPARγ gene was proven to be associated with complex T2DM (Andersen JG, 2001). Clinical studies have shown that presence of proline in residue 12 of PPARγ was associated with decreased sensitivity to insulin (Andersen JG, 2001). Moreover, HNF4 (MODY1) gene also showed an association of a frequent polymorphism in the P2 promoter with complex T2DM (Silander K, 2004); the frequent E23k variant in the KCJN11 gene encoding the ATP-sensitive K-channel subunit Kir6.2 has recently been associated with an increased susceptibility to T2DM in a few populations (Gloyn AL, 2004).

In short, recent discovery on the role of calpain 10, PPARγ, Kir6.2 and some other genes in the pathogenesis of complex T2DM contributes significantly to the understanding of the genetic background of this disease and its pathophysiological mechanisms. With the completion of the Human Genome Project and almost unlimited access to highly informative markers and extremely high output laboratory techniques, it will be much easier to further identify genes of interests. *Figure 5* below shows the genes that are responsible for the variable phenotypes including the earlier discussed monogenic forms and the polygenic/complex form as just explained. It is an interesting note that on the spectrum of complex polygenic T2DM, the role of environmental factors becomes more dominant than the role of genes.

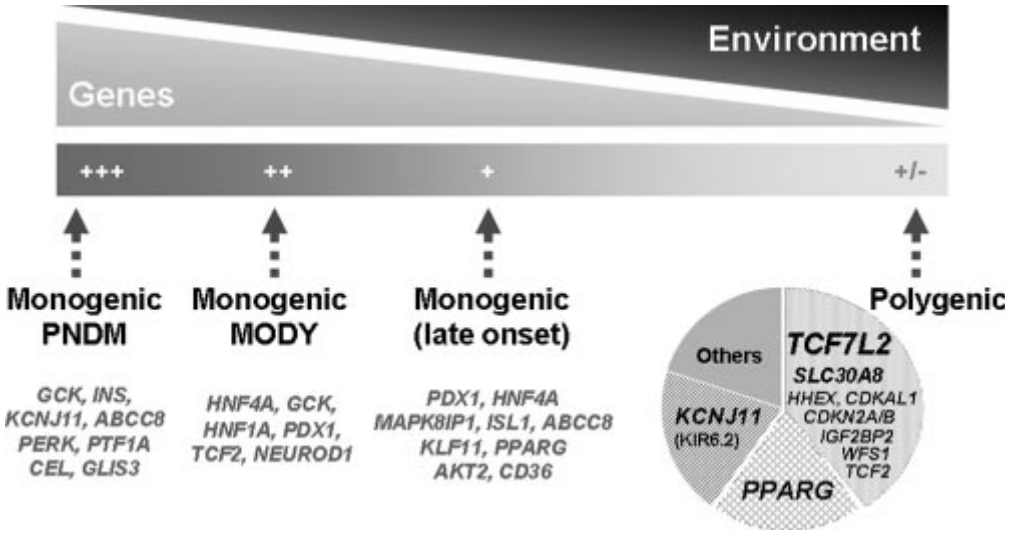

Figure 5: The concept of diabetes spectrum with the genes responsible for the variable phenotypes. *From left to right: genes shown above are associated with the variable phenotypes including the rare monogenic phenotype, or the more common polymorphisms. Mutations in these genes were shown to modulate disease risk and are linked to a clinical presentation of the different phenotypes of T2DM (Vaxillaire M, 2008).*

Genes in Pancreatic ß-cells

Some potential biological candidate genes involved in ß-cell development, differentiation and regeneration and the regulation of insulin gene transcription and the glucose sensing-insulin secretion coupling machinery have been identified. Table 1 below summarizes the hierarchy of transcription factors involved in development and differentiation of the ß-cell via the studies using knockout mice together with the studies of human MODY (Hansen L, 2005).

TOP LEVEL	WHOLE PANCREATIC MORPHOGENESIS
Notch Delta-like gene 1 RBP-J κ Hes-1 Hlxb9 ISL-1 IPF-1	
Intermediate Level Cdk4 HNF-6 Pax6 Pax4 Nkx2.2 Neurogenin3 NeuroD	Endocrine Differentiation and Islet morphogenesis
Lower Level IPF-1 NueroD HNF-1α HNF-4α HNF-3ß HNF-1ß	Transcription of, e.g. insulin and glucokinase genes

Table 1: Hierarchical organization of pancreatic ß-cell transcription factors. *These genes are suggested by knockout experiments in mice and they are also found to be involved in human diabetes (Hansen L, 2005).*

Based on the table above, it has been suggested that a top level of genes involved in ß-cell function includes members of the notch-signaling pathway, delta-like gene-1, RBP-J κ and Hes-1. In addition, homeodomain transcription factors: Hlxb9, IL-1 and IPF-1, control exocrine and endocrine development of the pancreas are also involved. Mutations in notch ligand jagged1 (jag1) as shown in the left part of *figure 6* cause Alagille's syndrome, in which pancreatic malformations and diabetes are often present (Krantz ID, 1998).

The intermediate level includes various transcription factors as shown in table above and *figure 6* below, such as HNF-6, Pax 6 and so on (Smith SB, 2000). Moreover, this level also includes a cyclin-dependent kinase Cdk4 that regulates endocrine differentiation and morphogenesis (Hansen L, 2005).

The lower level of this hierarchy consists of IPF-1, NeuroD, HNF-1α and so on that regulate genes, which are crucial for transcription of the insulin gene and the glucose sensing (GLUT2, glucokinase) of the differentiated ß-cells as shown on the right part of *figure 6* (Hansen L, 2005).

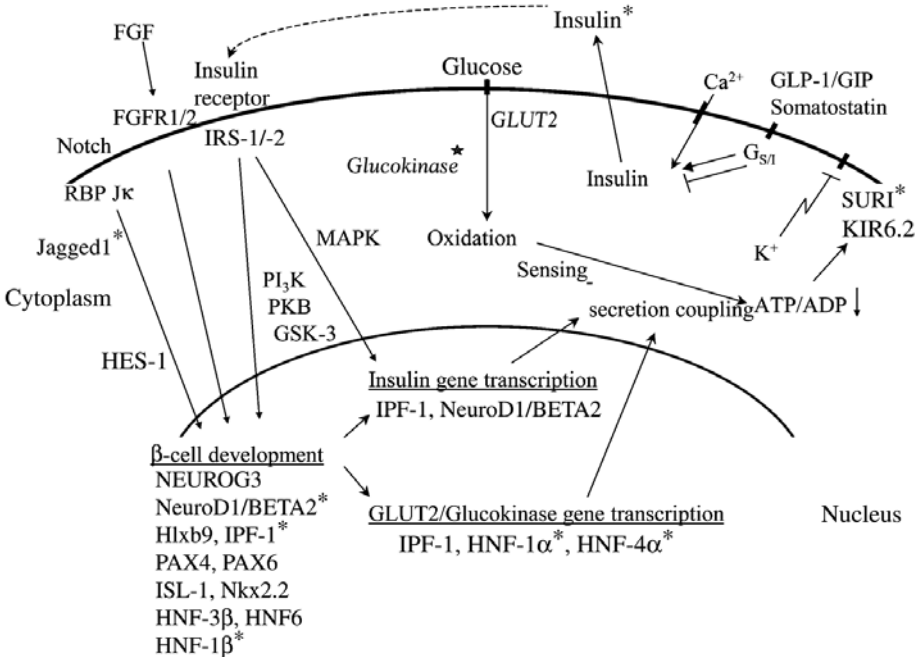

Figure 6: Gene involved in pancreatic ß-cell development, insulin gene transcription and insulin secretion in the cytoplasmic and nuclear compartments. *Left: pathways transmitting signals generated at the plasma membrane through the cytoplasm and resulting in the nuclear activation of specific transcription factors regulating ß-cell development, differentiation, insulin gene transcription, GLUT2 gene transcription and glucokinase gene transcription. Right: glucose and ligand regulated insulin secretion machinery. Top: insulin is suggested to act directly on ß-cells via an autocrine feedback loop. '*' means the ß-cell genes that have been identified to be directly involved in human insulin secretory disorder and diabetes mellitus (Hansen L, 2005).*

Other candidate genes in the ß-cells consist of genes directly involved in the functioning of these cells. Some of the genes have already been mentioned earlier. This class of the genes includes: insulin gene and genes involved in the glucose sensing-insulin secretion coupling machinery (see *figure 6*) (Hansen L, 2005). The latter further includes the following genes: glucokinase, Kir6.2 ion channels, mitochondrial genes, mitochondrial glutamate dehydrogenase, mitochondrial transcription factor A, uncoupling protein2 (energy dissipating protein) and G-protein-coupled receptors for endogenous secretagogues (e.g. glucagon, GLP-1, or the ß-cell specific receptor for sulfonylurea secretagogues) (Lynn FC, 2001).

Complications/Limitations in Gene Studies

Based on the genetic discoveries that are mentioned above, it is clearly shown that there seems to be a paucity of findings of genes involved in insulin resistance relative to ß-cell function: among the identified 38 individual susceptibility loci for T2DM, the majority code for proteins involved in ß-cell function, including glucose-sensing, proinsulin processing, and insulin secretion (Petrie JR, 2011). On the basis of these genetic discoveries, doubts are raised regarding the role that insulin resistance plays in T2DM pathophysiology, which might be less important than previously thought, moreover its role might be of a less critical one than impaired insulin secretion. Therefore, it is worth discussing that whether the classical understanding of T2DM pathophysiology should be revised and more focus placed on the ß-cell in the development of therapies for T2DM, or more emphasis should be given to the extent of difficulties in identifying insulin resistance genes reflecting limitations on study design, inadequate physiological assessment of insulin resistance or the complex underlying pathophysiology of insulin resistance (i.e. multiple parallel compensatory pathways).

There are several possible reasons why insulin-resistant genes are less represented in the existing genetic studies. Firstly, insulin action may have a smaller effect size, thus, it requires larger cohorts to discover the related genes. Studies have shown that the percentage of genes involved in insulin action is greatest in the largest study, even so, there are still

notably more T2DM genes linked to insulin secretion than insulin resistance. In addition, two intrinsic difficulties with the case control study approach may be the other reasons:

First of all, absence of diabetes is the only criteria to select the control population, which are likely to be drawn from a population across a spectrum of insulin resistance. Eventually, the power of any studies aiming to discover insulin resistant genes in the cases will be decreased due to the inclusion of insulin resistant controls (Petrie JR, 2011).

Secondly, the degree of ß-cell decompensation is a necessary indicator of diabetes diagnosis; therefore, it is rather logical that this approach will identify ß-cell function associated genes more readily than insulin resistance. In addition, it remains possible that the paucity of insulin resistance genes found by GWAS may be at least in part explained by the relative difficulty of accurately measuring small variations in insulin sensitivity compared to measuring small changes in insulin secretion in large populations (Petrie JR, 2011).

Therefore, it cannot yet be concluded that insulin resistance plays a less important role in comparison to impairment in ß-cell function in the pathophysiology of T2DM since many limitations are associated with the currently applied genetic tools such as GWAS as described above. Hence, better approaches are necessary to further investigate insulin resistant genes and evaluate their function in comparison to ß-cell related genes, only then it is possible to make a more definite conclusion.

In addition to the complications in the genetic discoveries of T2DM, there are also limitations in the techniques regarding data analysis of this complex disease. In fact, these limitations are general considering genetic studies because of the additional levels of regulation of translation and post-translational modifications, signal transduction and metabolic pathways in target tissue cells. Therefore, it is necessary to focus on the sensitivity and throughput of proteomic technologies as well, so to provide a broader picture to the pathogenesis of T2DM via the application of these techniques in the future.

Implications of gene studies into potential T2DM prevention and treatments

Genetic Screening for Prediction and Prevention

As a result of the rapid advances in genetic approaches, it will be possible to implement genetic screening into prediction and prevention of T2DM in the future. As mentioned earlier, the effectiveness of current type 2 diabetes management would be greatly improved if it is started at an earlier stage of the disease; therefore, if genetic testing could be used to predict type 2 diabetes, preventive measures could be taken and this disease could potentially be managed more easily. However, this is not an easy task since there are many variants associated with T2DM and the ones that have been identified so far only explain a small percentage of the total genetic variations that is thought to be present (Lango H, 2008). Hence, much more work needs to be accomplished before performing accurate predictive genetic testing:

Firstly, many more common genetic variants are expected to be identified by performing GWAS in different populations and by improving their power and coverage (Wolfs MGM, 2009). Secondly, performing thorough analyses of the genomic regions that show association to the disease by resequencing large numbers of patients and controls may identify genetic variants that have higher odds ratios than the common genetic variants identified so far (Wolfs MGM, 2009). Nonetheless, this is a laborious and costly task since many variants have to be tested for each subject, but hopefully, via improved genotyping and resequencing technologies, screening for such variants will be feasible in the near future.

New Insights into Intervention

Although the exact functions of most genes associated with T2DM are still elusive, they provide some potential insights into new targets and possibilities for novel interventions and personalized treatments. If certain genes are involved in the same molecular pathway or

physiological process, the entire pathway or process together with these genes would become a potential target for new anti-diabetic drugs. For instance, in combination with genetic screening, such information could be used to optimize diabetes management by prescribing drugs that act on those pathways that are affected in a patient.

Actually, improved drug treatment in diabetes as a result of these genetic studies can be seen in the current management of various MODY subtypes, which as explained before, are caused by mutations in genes involved in ß-cell signaling and/or growth in the pancreas, such as genes that are associated with both monogenic (ABCC8 and KCNJ11) and complex diabetes (KCNJ11, KCNQ1) (Hattersley AT, 2006). After the identifications of these mutations, the management of these diseases was greatly improved by the use of sulfonylurea derivatives, which enhances insulin secretion by targeting at ß-cell K-channels instead of exogenous insulin (Hattersley AT, 2006). Fortunately, MODY patients have been showing good response to sulfonylurea treatment, which is a perfect example of personalized therapy based on genetic screening.

Another good example is the targeting gene PPARγ, which is found through a candidate-based association study and likely to act in the pathway targeted by thiazolidinediones as explained earlier in the section of current treatment. No relationships are known between current medications and all the other genetic variants identified so far on the physiological level, so all these genes represent new potential targets for intervention.

Genes containing variants associated with T2DM that are involved in pancreatic growth and development present possibilities for promising intervention targets. Variants in these genes are usually related to the ß-cell population when there is an increased demand for insulin secretion and these genes or molecular pathways are possible targets for intervention aiming to correct the poor response of the ß-cells and ultimately improving insulin secretion (Wolfs MGM, 2009). Moreover, these variants are correlated with pancreatic damage, which would lead to endocrine malfunction of the pancreas at an early stage of the diabetic development. The possibilities for therapeutic intervention would largely depend on the severity and reversibility of such a malfunction.

A new area of potential intervention targets lies upon the involvement of mutations in the genes encoding mitochondrial proteins discovered by studies of MELAS syndrome. It is suggested that compromised mitochondrial metabolism may be a primary pathogenic event in the β-cells impairment and these genes might not only be involved in rare monogenic forms of diabetes, but certainly also for the more common form of T2DM. Therefore, these candidate genes are definitely becoming major interests of possible intervention development.

In addition to targets related to ß-cell function, the finding of ENPP1 gene which correlates insulin resistance and early onset of T2DM provides insights into potential disease prevention. The other gene RBP4 could be used for risk of assessment of T2DM since systemic insulin resistance is observed if this gene is elevated and further that RBP4 may play a causal role in insulin resistance and represent promising therapeutic target.

The role of microRNAs in T2DM

A new class of endogenous regulatory RNAs, microRNAs (miRNAs) may as well be a potential therapeutic target for the future. MicroRNAs are small non-coding RNA molecules of 21 to 23 nucleotides that regulate gene expression (Bartel DP, 2004). It has been realized that miRNAs, which function as translational repressors are important regulators of key biological processes and essential target tissues including pancreatic ß-cells and various insulin-target tissues. *Figure 7* (Guay C, 2011) below summarizes the characteristics and the functional roles of the miRNAs that are associated with the development of diabetes.

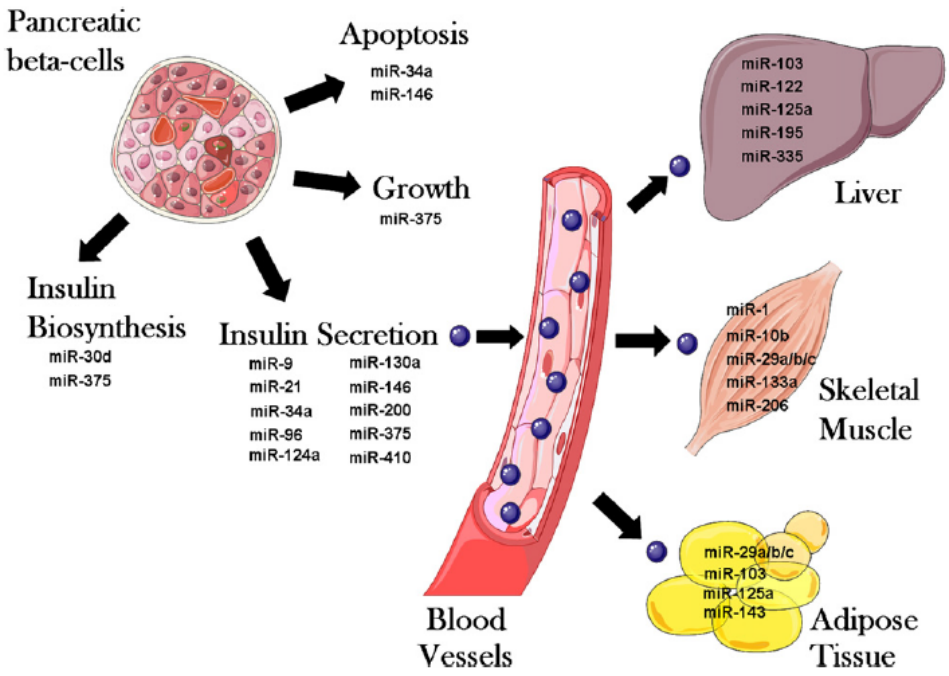

Figure 7. Overview of the miRNAs involved in the regulation of the functions of pancreatic ß-cells or insulin-target tissue in the context of diabetes. *As presented in the figure, different miRNAs are involved in the regulation of different biological functions of targeting tissues such as skeletal muscle, adipose tissue, etc. The figure depicts the importance of miRNAs in proper pancreatic islet development and function and the insulin-related targeting tissues (Guay C, 2011).*

Growing evidence indicates that miRNAs are involved in the pathogenesis of diabetes via their function in exocytosis (Guay C, 2011), which is the final step in the insulin secretory pathway (see *figure 1* in the appendix). It is found that miRNAs directly or indirectly control the expression of key components required for ß-cell exocytosis associated either with insulin containing secretory granules or with the plasma membrane (see *figure 2* in the appendix) (Guay C, 2011). Several studies have shown that deregulation of miRNA functions might be linked to diabetes. However, whether the disease is a direct cause of altered miRNA expression or if this altered expression occurs as a consequence of the pathological state is still unknown. Nonetheless, restoration of miRNA functions to normal levels is an attractive therapeutic strategy and could be used as tools for gene-based therapy to treat T2DM.

In addition to the role of miRNAs in ß-cell function, recent studies reported the detection of miRNAs in blood and other body fluids (Scholer N, 2010). Levels of miRNAs in serum samples from humans and other animals were stable, reproducible, and consistent among individuals. It has been discovered that diabetic patients and healthy people seem to have different miRNA expression profiles and plasma miRNAs seem to vary according to healthy or disease state (Scholer N, 2010). Therefore, miRNAs could be potential biomarkers that contribute to early detection of T2DM in patients, which allows more effective treatments at the early stage of the disease.

Conclusion and Discussion

Type 2 diabetes mellitus is an extremely complex disease with a tremendous social and economical burden. Patients that suffer from T2DM have a reduced quality of life and decreased life expectancy. Current treatments of T2DM are limited to relieving the symptoms and managing disease progression, but not aiming at the actual pathophysiology of the disease. Recent advances in genetic studies have contributed substantially to the understanding of the etiology of this complex disease, identifications of susceptibility genes and provided many

interesting and potential targets for novel therapeutic interventions. Some of the targets are already used for a more personalized treatment and have showed promising results. These progresses give great hope for the future and increasing confidence in the techniques being employed to further increase our knowledge in the field of human genetics of this multi-factorial disease and its potential therapeutics. Therefore, it is believed that with the rapid development of genetic research tools, the mechanisms underlying the biological processes of this disease will be further dissected and innovative ways to intervene them will be developed. *Figure 8* Freeman H, 2006) below summarizes some of the key genes associated with both monogenic and complex diabetes that have been discussed in this paper.

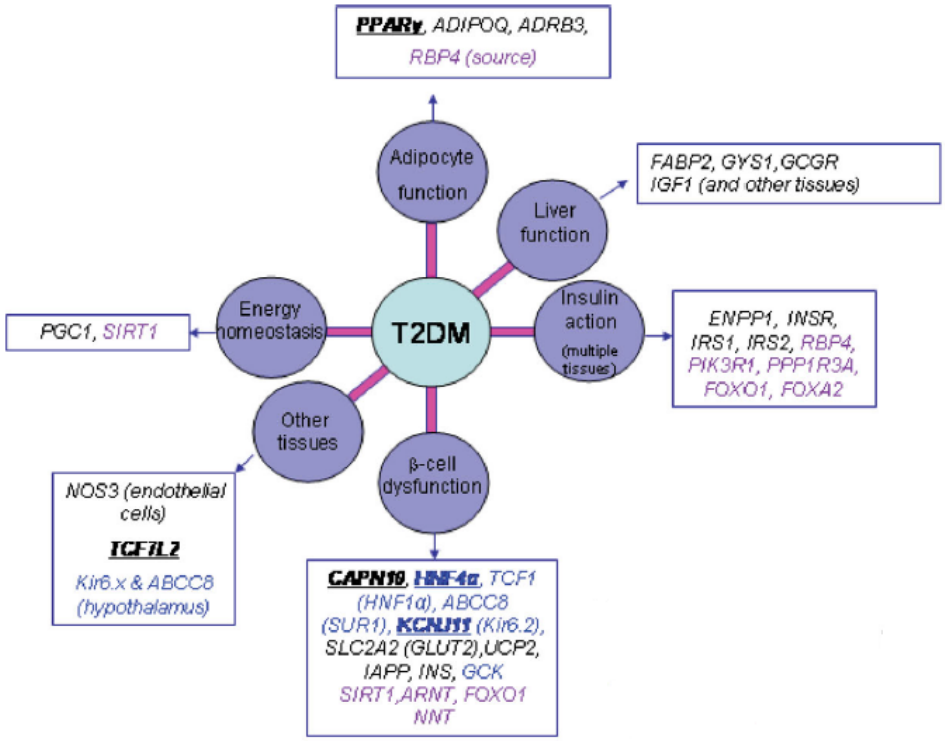

Figure 8: Genes involved in T2DM. *Genes that are shown in the figure are the most promising ones from animal models and human association studies. Genes in bold and underlined are the best supported by human genetic data (Freeman H, 2006).*

In addition to the genes depicted in *figure 8* above and the ones have been discussed in this thesis, there are more T2DM related genes that are not covered here. Based on these scientific findings, genetic factor is not the only determinant, the interaction of environmental and genetic factors, and the degree to which they interact, should not be underestimated regarding disease development. The multi-faceted approaches to understanding this complex disease together with cheap and fast genotyping as well as development of novel genetic-statistical tools already helped and will keep leading us to define further the complicated web of genetic and environmental factors contributing to T2DM pathogenesis. It is important to realize that as the roles that dietary and life-style choice play in this complex disease are understood better and better, genetics therapy alone is not sufficient to target this complex disease effectively. Therefore, personalized life-style managements and genetic therapies should be applied to T2DM patients simultaneously. The future T2DM treatments are believed to be no longer a single option, but will tackle several aspects of this disease at the same time.

These combined efforts will speed up molecular genetics driven developments of antidiabetic therapeutics and diabetes prevention. With regard to miRNAs, measurements of the level of specific miRNAs may become useful tools to identify individuals at risk for

developing T2DM hopefully preventing the development of the disease or more efficient disease management since the earlier diagnosis. The fact that output from genetic studies is already integrated into the treatment of monogenic T2DM, it is believed that more and more new therapeutic strategies will definitely be developed continuously.

Appendix

Figure 1: Mechanism of insulin release.

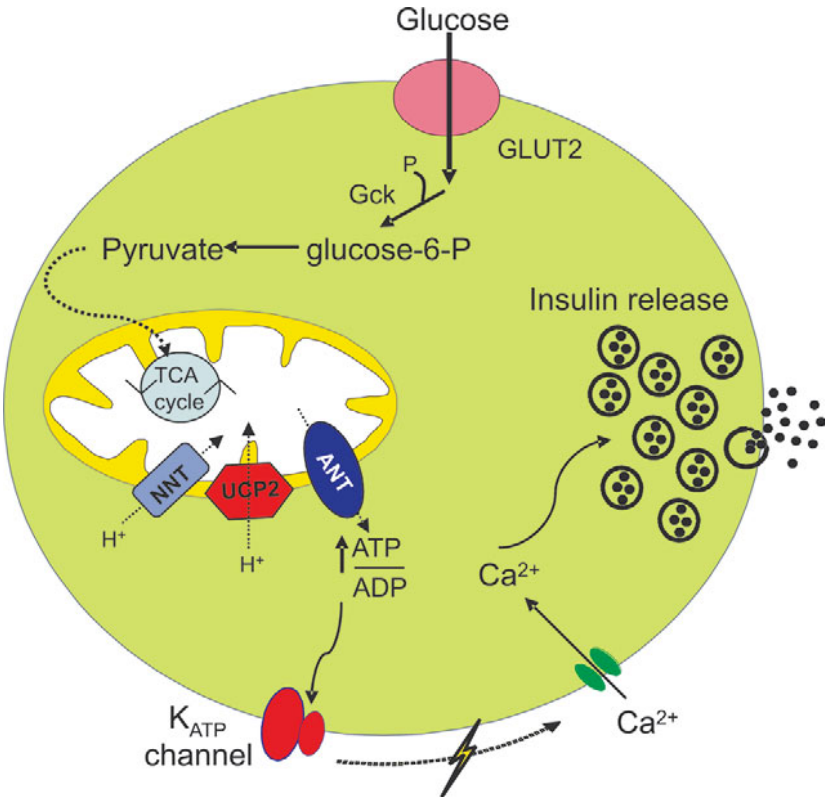

Figure 1: Mechanism of insulin release. Changes in beta-cell metabolism are coupled to changes in insulin release via metabolic regulation of the activity of the ATP-sensitive potassium (KATP) channel. When glucose levels rise, increased beta-cell metabolism produces elevation of cellular ATP levels. This causes closure of K_{ATP} channels, which leads to membrane depolarization, activation of voltage-gated Ca^{2+} channels, Ca^{2+} entry and a rise in $[Ca^{2+}]$ that triggers insulin secretion (Freeman H, 2006).

List of novel T2DM-susceptibility loci: (Petrie JR, 2011)

a) SLC30A8 (encoding a zinc transporter and member of solute carrier family, potentially linked with beta cell insulin secretion;
b) A haplotype block encompassing the HHEX/IDE/KIF11 (haematopoietically expressed homeobox/insulin-degrading enzyme);
c) CDKAL1 (cyclin-dependent kinase 5 regulatory subunit associated protein-like 1), this is a widely expressed gene with sequence similarity to cell-cycle regulatory proteins;
d) A region near CDKN2A/CDKN2B (cyclin-dependent kinase inhibitor 2A and 2B respectively), genes regulating cell cycle progression;
e) IGF2BP2 [IGF-2 (insulin-like growth factor-2)-mRNA-binding protein 2], encoding a protein regulating the translation of IGF-2;
f) FTO (fat mass and obesity-associated), via its effect on BMI (body mass index). FTOhas been identified as a member of the Fe(II)- and 2-oxoglutarate-dependent oxygenase family, which may catalyse the demethylation of single-stranded DNA and is highly expressed in the CNS; however, its functional role in mediating obesity risk remains unknown.

Figure 2: Regulation of insulin exocytosis by miRNAs.

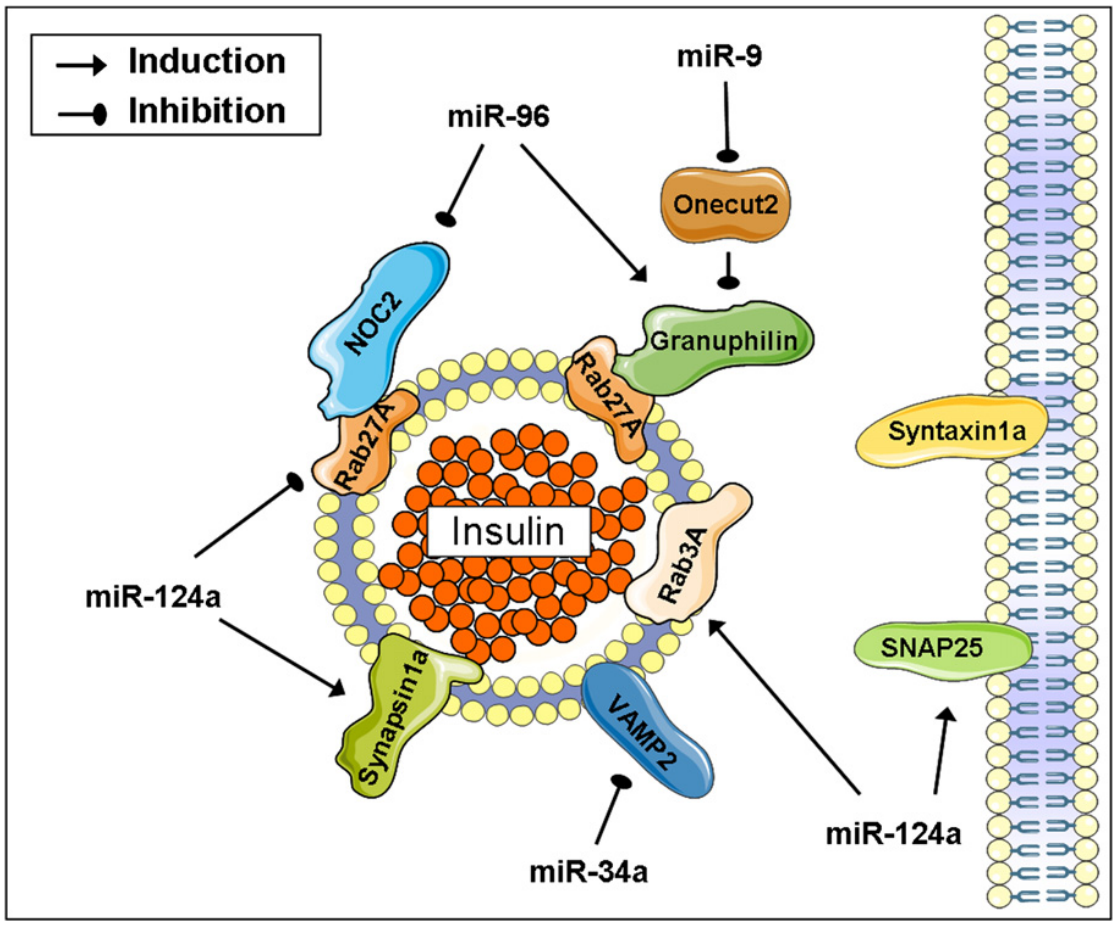

Figure2: Regulation of insulin exocytosis by miRNAs. The figure depicts the miRNAs that directly or indirectly control the expression of key components required for beta-cell exocytosis associated either with insulin containing secretory granules or with the plasma membrane (Guay C, 2011).

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